

9. Reduce pressure and allow liposomes to anneal at 44° C. for 30 min.
10. Add 10 ml of warm (50°–52° C.) 0.1 molar sulforhodamine B to vessel and mix.
11. Extrude the warm liposome preparation through a 0.4 micron then a 0.2 micron Biorad Unipore polycarbonate membrane (Biorad #313-0059 and #313-5059, respectively).
12. Dilute liposomes to a total volume of approximately 80 ml in a 90 ml ultracentrifuge tube using sodium acetate/saline buffer, pH 4.5.
13. Centrifuge at 75,000 Xg for 30 min.
14. Resuspend pelleted liposomes to 80 ml with sodium acetate/saline buffer, pH 4.5.
15. Repeat #13 and #14, then #13 again.
16. Resuspend pelleted liposomes in 10 ml Tris buffer, pH 8.0 (50 mM Tris, 100 mM NaCl, 1 mM EDTA, 310 mOs/kg).
17. Hold at 4° C. until protein reaction.

#### EXAMPLE IA

##### Preparation of

Distearoylphosphatidylethanolaminemaleimidophenylbutyrate used in Example I

Distearoylphosphatidylethanolamine (119.2 mg, 0.1593 mmol, Avanti Polar Lipid) was suspended in 30 ml of chloroform and heated to reflux under a nitrogen atmosphere until all solid had dissolved. The solution was allowed to cool to room temperature followed by the addition of triethylamine (22.2 ul, 0.1593 mmol, Aldrich) and succinimidyl-4-(p-maleimidophenyl) butyrate (79.45 mg, 0.2230 mmol, Pierce). The reaction mixture was allowed to stir overnight at room temperature under a nitrogen atmosphere. The mixture was concentrated under reduced pressure to yield a pale yellow waxy solid (270.7 mg) that appeared as one major spot and several minor spots upon TLC analysis (silica, 65:25:4 CH<sub>2</sub>Cl<sub>2</sub>:CH<sub>3</sub>OH:H<sub>2</sub>O). The spot was visualized with UV light and Molybdenum Blue Spray Reagent (Sigma), R<sub>f</sub> 0.5

The crude product was chromatographed on four silica gel, preparative, thick-layer plates (E. Merck, 2.0 mm) developing with 65:25:4 CH<sub>2</sub>Cl<sub>2</sub>:CH<sub>3</sub>OH:H<sub>2</sub>O. The upper band of the two Molybdenum Blue active bands was isolated and the product extracted with 50% CH<sub>2</sub>Cl<sub>2</sub>:C<sub>2</sub>H<sub>5</sub>OH. Evaporation of the solvent afforded the product as a white solid (65.75 mg).

IR (Neat): 2910(s), 2845(s), 1734(s), 1715(s), 1510(m), 1460(m), 1390(m), 1370 (mw), 1242(m), 1230(m), 1100(m), 1060(m), 905(m), 820(m), 685 cm<sup>-1</sup>(m).

The liposomes prepared in this manner include rhodamine dye and may be sensitized with a ligand by procedures known in the art to produce a tracer for use in the present invention.

The following Example II illustrates the preparation of tracer by sensitizing the liposome with an antibody.

##### Sensitizing Liposome (Example I) with Antibody to Produce Tracer

#### EXAMPLE II

1. To 8 mg protein A purified antibody, add 0.4 ml 1 M dithiothreitol in sodium acetate/saline buffer, pH 4.5.
2. Vortex and let react 30 min at room temperature in the dark.
3. Remove dithiothreitol by passing the reaction volume over a Sephadex G-25 medium column equili-

brated with Tris pH 8.0 buffer (50 mM Tris, 100 mM saline, 1 mM EDTA, 310 mOs/kg).

4. Monitor the O.D. 280 and pool void volume fractions.
5. Mix this solution with the 10 ml of freshly prepared liposomes.
6. Flush with N<sub>2</sub> and seal.
7. React overnight at room temperature.
8. Wash twice, by centrifugation, these protein-labeled liposomes using the standard Tris buffer.
9. After last wash, resuspend pellet in 40 ml Tris.
10. Store at 4° C.

#### EXAMPLE III

##### 15 Nitrocellulose Disc Immunoassay for HCG (Pregnancy Test)

##### Reagents

1. Adsorption Buffer 5: BD, Catalog #614335
2. HCG antibody to the alpha-chain of hCG
3. Nitrocellulose Paper: Schleicher & Schuell, ME 25, 0.45 um porosity
4. Bovine Serum Albumin: Sigma, Catalog #A-7906
5. Urine Controls: BDI, Catalog #255815
6. Tracer: Liposome prepared by method of Example I and sensitized with antibody to the B chain of hCG by the method of Example II.

##### Procedure:

1. Cut 1 cm disc of nitrocellulose paper.
2. Pipet 3 ul of 1:50 dilution of hCG antibody (dilution made in AB5) to the center of disc.
3. Allow to dry at room temperature 15 minutes.
4. Pipet 300 ul of 5% BSA in AB5 (filtered through 0.45 micron filter prior to use) to each disc.
5. Incubate disc 1 hour at 37° C.
6. Decant liquid.
7. Pipet 200 ul of urine control or urine.
8. Incubate 1 hour at room temperature.
9. Decant control or urine.
10. Wash disc twice with 1.5 ml AB5.
11. Pipet 300 ul of 1:12 dilution of tracer (dilution made in AB5) to each disc (stock liposomes contain about 1μ mole lipid/ml)
12. Incubate 1 hour at room temperature.
13. Decant tracer.
14. Wash twice with 1.5 ml AB5.
15. Visible spot when viewed under UV light is positive for pregnancy.

#### EXAMPLE IV

##### Preparation of

Distearoylphosphatidylethanolamine-Digoxigenin

- Distearoylphosphatidylethanolamine (400.0 mg, 0.5346 mmol, Avanti Polar Lipid) was suspended in 50 ml of CHCl<sub>3</sub>:CH<sub>3</sub>OH (9:1) and heated to reflux under a nitrogen atmosphere until all solid had dissolved. The solution was allowed to cool followed by the addition of 3-ketodigoxigenin (207.7 mg, 0.5346 mmol) and 2.0 g of 4A sieves (Sigma). The reaction mixture was allowed to stir at 60° C. for 3 hr. under a nitrogen atmosphere at which time sodium cyanoborohydride (36.95 mg, 0.5881 mmol, Sigma) was added. The mixture was then allowed to stir at room temperature overnight. The reaction was filtered and concentrated under reduced pressure to yield a white foam (579.6 mg) that appeared as one major spot and several minor spots under TLC analysis (silica, 20% CH<sub>3</sub>OH:CH<sub>2</sub>Cl<sub>2</sub>). The spot was